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Small mammal inventory in the Lama forest reserve (south Benin), with new cytogenetical data

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Abstract. The Lama forest is the largest natural forest in southern Benin, and one of the last remnant forests within the Dahomey Gap. It harbours several species of major importance in terms of conservation. Small mammals are known to represent more than 80% of the African mammalian species diversity but they have received little attention in Benin. In this article we present the results of the first terrestrial small mammal species inventory (murid rodents and shrews) in the Lama forest. In September and October 2007, we captured 280 small mammals belonging to 12 species, identified by morphological and genetic analysis. We also provide detailed cytogenetic data for six of the 12 captured species. For five of them, we compare our data with previously published karyotypes, and for the sixth one (*Hylomyscus pamfi*), the karyotype is published here for the first time. Two of the captured species are closed-forest specialists (*Praomys misonnei*, *H. pamfi*), and *H. pamfi* is endemic to the Dahomey Gap region. Our results are congruent with those obtained on other animal groups, and highlight the importance of the Lama forest for the conservation of the country's forest biodiversity.

Key words: Dahomey Gap, karyotype, rodents, shrews, species inventory, West Africa

Introduction

The Lama forest reserve is situated in the so-called Dahomey Gap, a savannah corridor that currently separates Upper Guinean (West Africa) and Lower Guinean (western Central Africa) rain forest blocks (Demenou et al. 2018). It is the largest natural forest in southern Benin, and one of the last remnant forests within the Dahomey Gap (Ballouche et al. 2000, Nagel et al. 2004). It harbours several species of major importance in term of conservation. The flagship species of the Lama forest is the red-bellied monkey (*Cercopithecus erythrogaster erythrogaster*). The red-bellied monkey is listed as Endangered on the IUCN red List, and the subspecies inhabiting the Lama forest is endemic to the Dahomey Gap. Due to hunting and habitat

destruction, surviving populations now seem to be restricted to only a few forest patches, and its main population occurs in the Lama forest (Sinsin et al. 2002, Nobime & Sinsin 2003, Houngbédji et al. 2012). Several rare and threatened forest ungulates species, including the sitatunga (*Tragelaphus spekii*), the royal antelope (*Neotragus pygmaeus*), the black duiker (*Cephalophus niger*) and the yellow-backed duiker (*C. silvicultor*), are found in the Lama forest (Kassa & Sinsin 2003), and 15 forest bird species in Benin have been observed only in the Lama forest thus far (Waltert & Mühlenberg 1999). If the large mammal and bird species diversity of the Lama forest is well known, this is not true for all taxa. However, several inventories on various species of arthropods were carried out during the last 20 years (Fermon et al. 2001, Ullenbruch 2003, Attignon et

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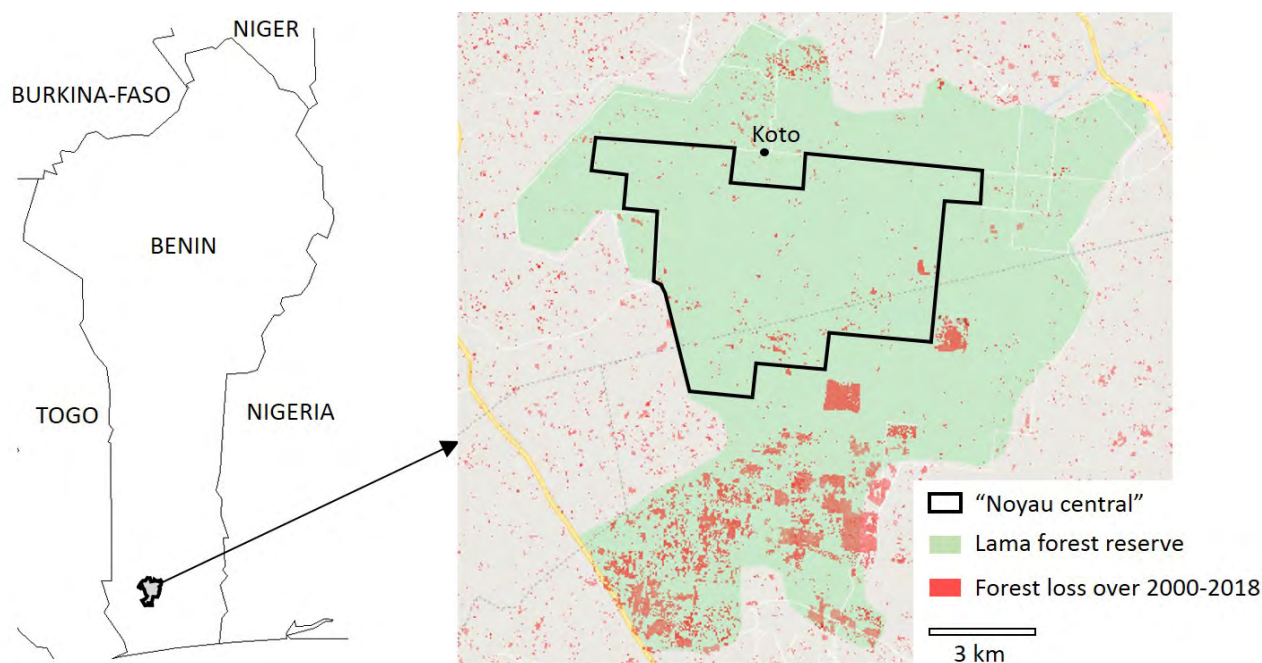


Fig. 1. Maps showing the location of Lama forest reserve in Benin (right), the boundaries of Noyau Central, the location of Koto village and forest cover loss 2000-2018 based on online data (Hansen et al. 2013; left).

al. 2004, 2005, Lachat et al. 2006a, b, 2007), as well as on the herpetofauna (Ullénbruch 2003, Piquet et al. 2012). Preliminary evidence was found that the Lama forest and probably other forest remnants of the Dahomey Gap are home to endemic beetle and butterfly species (Goergen 2003), and several rare species of ground beetles (Carabidae) were observed in the Lama reserve (Nagel 2003). A new chameleon species and probably also a new gecko species (Ullénbruch 2003, Nagel et al. 2004) were also recorded. Finally, more than 15 species of plants of the Lama forest are listed on the Red List of threatened plants (Nagel et al. 2004). All these examples underline the importance of the Lama reserve, the largest remnant of dense semi-deciduous forest in Benin, and its characteristic fauna for biodiversity conservation in Benin.

Small mammals are known to represent more than 80% of the African mammalian species diversity (Denys et al. 2001). However, despite their importance in terms of species diversity and their importance in the functioning of forest ecosystems, they have received little attention in Benin. Moreover, rodents represent a considerable source of animal protein and income for villagers; as they are the animal species most frequently consumed and preferred by villagers near the Lama forest reserve (Codjia & Assogbadjo 2004, Assogbadjo et al. 2005). Consumption of rodents is primarily of grasscutters and giant rats, but eight other species are also occasionally eaten. To

date, no reliable rodent or shrew species inventory has been carried out in the Lama forest. The only available data for Benin are those published by Green (1983), Robbins & Van Der Straeten (1996), Bergmans (1999), De Visser et al. (2001), Granjon et al. (2005), Durnez et al. (2010) and Nicolas et al. (2010a).

The aim of this study is to catalogue the species diversity of small terrestrial mammals in the Lama forest reserve, and to provide cytogenetic data on some of these species. Results should allow us to better judge the importance of this forest reserve for the conservation of small mammal forest species in Benin.

Material and Methods

Study sites

The Lama forest reserve is located in south Benin, between 6°55' and 7°00' N, and 2°04' and 2°12' E (Fig. 1). It is located in the Lama depression, which in the north is defined by the Zagnanado and Ketou plateaus and in the south by the Allada and Sakete plateaus. The Lama forest reserve extends over an area of about 162 km² and is divided into a fully protected central part, the Noyau Central (48 km²), a cordon of forest plantations (90 km²) and settlements (24 km²). Teak plantations enclose the Noyau Central almost entirely, forming a buffer zone that separates this region from surrounding cropland.

The altitude of the forest averages 60 m a.s.l. Clay-rich vertisols are the prevailing soils in the Lama forest (Attignon et al. 2004). The water network is exclusively composed of ponds and seasonal streams. Due to reduced water infiltration, vertisols may become seasonally waterlogged. The climate is subequatorial, with two rainy and two dry seasons. The annual average rainfall is 1100 mm. The highest rainfall occurs in June and the lowest in January. Relative humidity remains high throughout the year, with a maximum of 100% and a minimum of 50-60%. The annual average temperature varies between 25 °C and 29 °C, with a maximum in February and March (39 °C) and a minimum in December (15 °C) (Attignon et al. 2004).

The Noyau Central is composed of a mosaic of primary semi-deciduous forest, degraded forest of different successional stages and *Chromolaena odorata* thickets. Dominant tree species of the semi-deciduous forest are *Azelia africana*, *Albizia zygia*, *Anogeissus leiocarpus*, *Ceiba pentandra*, *Dialium guineense*, *Diospyros mespiliformis*, *Drypetes floribunda*, *Celtis brownie* and *Mimusops andongensis* (Nagel et al. 2004, Lachat et al. 2006a). Lianas such as *Flabellaria paniculata* are also common. The understorey vegetation can be dense throughout all seasons, and canopy height is approximately 20 m, with some emergent trees reaching 30 m.

Sampling methods

Sampling was performed in August and September 2007. Animals were trapped and handled under the Guidelines of the American Society of Mammalogists (Sikes et al. 2016).

Two kinds of devices were used to trap small mammals in the Noyau Central: (i) six traplines (each 500 m long) of 100 traps baited with palm nuts. Each trapline was set for six days and contained 50 Sherman live traps and 50 metal snap traps spaced at 5-m intervals (one Sherman and one snap trap alternately). (ii) two lines of dry pitfall traps with plastic drift fences (150 m long) comprising thirty 10-L plastic buckets (260 mm deep, 260 mm top internal diameter, 200 mm bottom internal diameter) placed at 5-m intervals (Nicolas et al. 2003). Small holes (3-5 mm) were burned in the bottom of each bucket to allow water drainage and to keep the animals alive until the bucket was checked. Pitfall lines were set for 11 days. These two types of arrays were used because preliminary studies showed that they

have complementary effects. Most murid species are preferentially captured by Sherman or snap traps, but shrews and some rare murid species are mostly captured by pitfall traps (Nicolas & Colyn 2006). All traps were checked daily.

Several Sherman traps were also given to the inhabitants of Koto village and placed in houses and crops around the village, which is located at the periphery of the Noyau Central.

Because of the existence of several sibling species, the identification of numerous small mammal species is not possible by external characteristics only. Thus, all specimens captured were euthanized and kept as voucher specimens at the Muséum National d'Histoire Naturelle (Paris, France). A previous study showed that removal trapping, with similar conditions to those employed in our study, did not adversely affect shrew richness or local population numbers in an undisturbed forest in Gabon (Nicolas et al. 2003). Tissue samples were preserved from all specimens in 95° ethanol for future molecular studies. Necropsies of muscular tissue of 11 samples were immediately soaked in a sterile physiological solution (D-MEM (Gibco)) with glutamax (Thermo Fisher Scientific), with 10% foetal calf serum supplemented with antibiotics and antifungal agents (final concentration: penicillin-streptomycin: 40 UI/ml; amphotericin B, 2.5 µg/ml; gentamicin, 1 µg/ml). They were stored at about 6 °C until transport to our laboratory in Paris, where cell cultures were initiated.

Species identification

Species identifications were based on external and/or crania-dental analysis, and were confirmed, for several specimens, by molecular analysis (mitochondrial 16S rRNA or cytochrome *b* gene sequencing, Table 1). The DNA sequences obtained were compared through a BLAST analysis to those available in the Genbank database. Recent molecular studies are available for the genera *Hylomyscus* (Nicolas et al. 2012, 2020), *Praomys* (Nicolas et al. 2012, Mikula et al. 2020), *Lemniscomys* (Nicolas et al. 2008, Denys et al. 2020), *Mastomys* (Nicolas et al. 2012) and *Rattus* (Lack et al. 2012, Colangelo et al. 2015), allowing accurate molecular species identification.

Molecular data for the subgenus *Nannomys* (Bryja et al. 2014), showed high cryptic diversity in this subgenus. Our specimen clusters unambiguously with MOTU27 of Bryja et al. (2014), i.e. *Mus* (N.)

Table 1. Number of specimens captured per species in Koto village and Noyau Central of Lama forest, and number of specimens included in genetic analyses (Cytb or 16S gene sequencing) for confirmation of species identification and in cytogenetic analyses.

	Koto village	Noyau Central	Cytb	16S	Cytogenetic data	
Sampling device	Sherman	Sherman + Snap traps	Pitfall traps			
Number of trap-nights	300	3600	660			
Soricidae						
<i>Crocidura fuscomurina</i>	-	1	10			
<i>Crocidura cf. olivieri</i>	11	-	-	7	8	
Muridae						
<i>Arvicanthis cf. rufinus</i>	-	1	-	1		
<i>Hylomyscus pamfi</i>	1	1	-	2	2	1
<i>Lemniscomys striatus</i>	2	-	-	2		1
<i>Mastomys erythroleucus</i>	-	-	2	2		-
<i>Mastomys natalensis</i>	5	1	1	7		1
<i>Mus (Nannomys) minutoides</i>	-	-	1	1		
<i>Praomys derooi</i>	4	-	-	4		1
<i>Praomys misonnei</i>	-	231	3	18	1	-
<i>Rattus rattus</i>	3	-	-	2		1
<i>Uranomys ruddi</i>	2	-	-	2	2	1
Total	28	235	17			
Trap success	9.33	6.53	2.58			

minutoides. This species is a widely distributed species in most of sub-Saharan Africa and has a strong phylogeographical structure. Our specimen clusters with the West African clade and represents the eastern known limits of this clade, otherwise known from Guinea to Ghana.

Recent molecular data showed that *Crocidura olivieri* is polyphyletic and divided into five geographical clades with allopatric or parapatric distributions (Jacquet et al. 2015). Our specimens cluster with clade II specimens of Jacquet et al. (2015) from West Africa. They are referred to as *C. cf. olivieri* in the present paper.

The taxonomy of the genus *Arvicanthis* is still under debate, and we compared our DNA sequence to those recently published by Bryja et al. (2019). Our *Arvicanthis* specimen clusters unambiguously with the species assigned to *A. rufinus* by these

authors. However, this species is composed of two distinct mitochondrial genetic clades with distinct cytotypes (ANI-2 and ANI-4). Our specimen clusters with the ANI-4 specimens. Given that the taxonomic status of these two clades deserves additional studies, we refer to our specimen as *A. cf. rufinus*.

No molecular study of the monospecific genus *Uranomys* is available. Thus, we simply compared our two specimens to the Genbank sequence Z83922.

Estimates of species richness and trap success

Rarefaction curves were constructed with the Software EcoSim v.7 to assess whether our trapping effort per site (i.e. Koto and Noyau Central) and sampling device was enough to obtain reliable estimates of species richness (Gotelli & Entsminger 2004). Trap success was defined as the number of

individuals caught per 100 bucket-nights or 100 trap-nights.

Cell cultures and cryopreservation

Tissue fragments were rinsed and explants were grown at 37 °C in a sterile physiological solution (DMEM high glucose with glutamax, with 10% foetal calf serum supplemented with antibiotics and antifungal agents (final concentration: penicillin-streptomycin, 20 UI/ml; amphotericin B, 1.25 µg/ml; gentamicin, 0.5 µg/ml). For each specimen with a successful culture, tissue fragments or cultured fibroblasts (6 million cells) were put in 2 mL cryovials with cryopreservative solution (DMEM with glutamax, with 10% foetal calf serum and 10% DMSO (Sigma)) and kept in liquid nitrogen. The specimens are stored in the TCCV collection (Tissus et Cellules Cryoconservés de Vertébrés), at the Paris Muséum National d'Histoire Naturelle, and are available upon request at: https://science.mnhn.fr/institution/mnhn/collection/tccv/item/search?lang=en_US.

Metaphase preparations and chromosome banding

For RBG banding (revealing early replicating DNA), FdU (fluorodeoxyuridine, Sigma, final concentration of 0.06 mg/ml) was used to synchronize cultures over 18 h. BrdU (bromodeoxyuridine, Sigma, final concentration of 0.02 mg/ml) was added to reinitiate cell proliferation and to be incorporated into replicating DNA for 7-8 h. For GBG banding (revealing late replicating DNA), BrdU (bromodeoxyuridine, Sigma, final concentration of 0.1 mg/ml) was used to synchronize cultures for 18 h. Thymidine (Sigma, final concentration of 0.06 mg/ml) was added to reinitiate cell proliferation and to be incorporated into replicating DNA for 6 h. For all preparations,

colchicine (Sigma, final concentration 0.04 mg/ml) was added for the two last hours. Hypotonic shock (foetal calf serum 1 vol. + distilled water 5 vol. and KCl at final concentration 0.93 mg/ml) was used for 10 min at 37 °C. Cells were fixed with Carnoy's fixative solution, spread on cold slides, dried and stored at -20 °C until further use. RBG, GBG and CBG banding, and Ag-NOR staining techniques were applied on slides (Dutrillaux & Couturier 1981, Popescu et al. 1998). Metaphases were karyotyped using the Ikaros3 software (Metasystems, Altussheim, Germany).

FISH and chromosome painting

For telomeric FISH labelling, a telomere probe (3'biotinylated 24-mer composed of four TTAGGG repeats with LNA modifications, kindly provided by Christophe Escudé, UMR 7196 MNHN-CNRS-INSERM-Sorbonne Université) was used at a final concentration of 0.3 µM in PBS. Chromosome preparations were denatured at 80 °C for 5 min then hybridized for 1 h at 37 °C on a denaturation/hybridization system (ThermoBrite TM, StatSpin, Abbott Laboratories, Illinois), and finally washed at 37 °C for 5 min in 2 x SSC, pH 7.

For chromosome painting, chromosome preparations on slides were denatured at 75 °C for 5 min, and the human X chromosome painting probe (3 µL of probe and 7 µl of buffer, Amplitech) was denatured at 70 °C for 10 min. The probe is loaded on the slide and hybridized for 48 h at 37 °C, then washed at 37 °C for 5 min in 2 x SSC, pH 7.

Hybridized probes (telomeric FISH and chromosome painting) were revealed in green by indirect detection with goat anti-biotin antibodies (10 mg/ml, Vector laboratories, Burlingame) followed by FITC-conjugated anti-goat antibodies

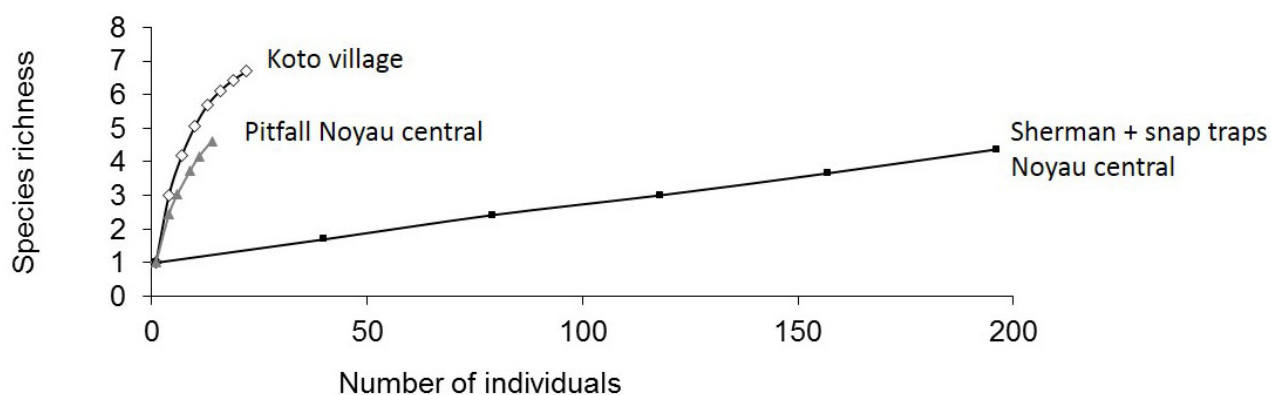


Fig. 2. Rarefaction curves per site (Koto village and Noyau Central) and trapping device.

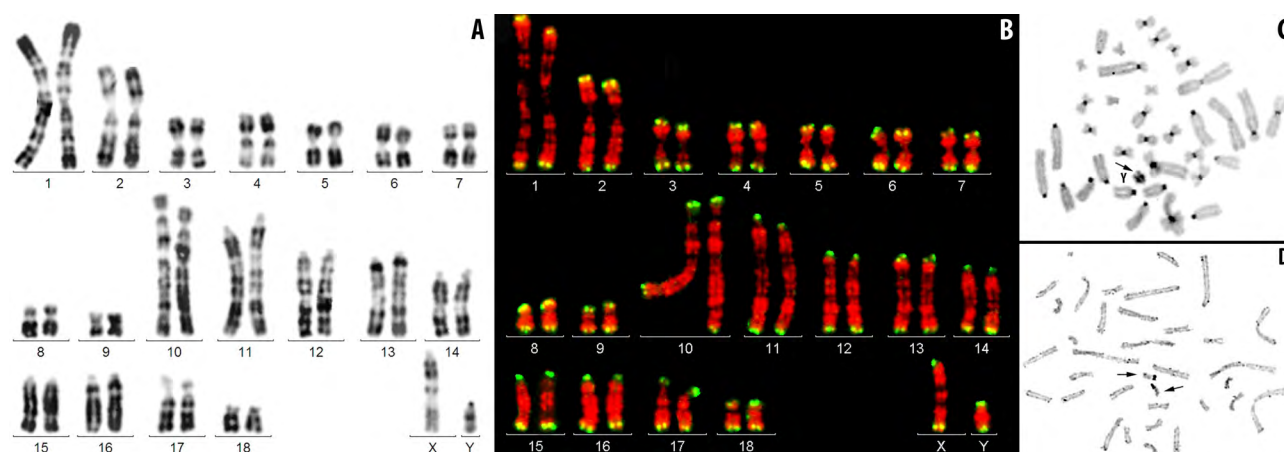


Fig. 3. Karyotype of male *Rattus rattus* MNHN-TCCV-857 (2N = 38, XY). A) With RBG banding. B) With telomeric FISH. C) Metaphase plate with CBG banding. Arrow indicates Y chromosome. D) Metaphase plate with Ag-NOR staining. Arrows indicate the NOR-carrier chromosomes 18.

(5 mg/ml, P.A.R.I.S., France). Chromosomes were counterstained with PI (propidium iodide, 0.3 mg/ml). Observations were performed with an epifluorescent microscope (DMRB, Leica Microsystems, Germany) and images were captured using a cooled CCD camera (ProgRes MS cool, Jenoptik, Germany) and treated using capture software (Isis, Metasystems, Altusheim, Germany). Chromosomes were identified using a computer generated reverse PI-banding.

Results

Species richness and trap success

A total of 280 small mammals belonging to two families (Soricidae and Muridae) and 12 species were captured (Table 1).

Of these, 252 specimens belonging to seven species were captured in the Noyau Central, with most captured with traplines. Rarefaction curves (Fig. 2) show that our results probably underestimate the number of species: no plateau is reached (particularly for the pitfall lines). This outcome is probably related to the fact that this community is characterized by the presence of one highly dominant species (*P. misonnei*: 93% of the specimens captured) and numerous rare species. In consequence, it would have been necessary to substantially increase our trapping effort in order to catch the rarest species.

A total of 28 specimens belonging to seven species were captured in Koto village. However, the rarefaction curve shows that our sampling did not represent total species richness. This is not surprising given the low trapping effort performed in Koto village (only 300 traps-nights). Trap success

varied from 2.58% in the Noyau Central with pitfall traps to 9.33% in Koto village with Sherman traps (Table 1).

Cytogenetics

A fibroblast proliferation, sufficient for cytogenetic analyses and cryopreservation was obtained for six of the 11 specimens (Table 1). The collection numbers for these six specimens are: MNHN-TCCV-857 (*Rattus rattus*), MNHN-TCCV-833 (*Uranomys ruddi*), MNHN-TCCV-839 (*Lemniscomys striatus*), MNHN-TCCV-861 (*Hylomyscus pamfi*), MNHN-TCCV-841 (*Mastomys natalensis*), MNHN-TCCV-856 (*Praomys derooi*).

The specimen MNHN-TCCV-391 (*Hylomyscus walterverheyeni*) from Gabon has been added to this analysis for comparison with *Hylomyscus pamfi*.

The karyotype of a male *Rattus rattus* has 2N = 38 chromosomes (Fig. 3). The X chromosome is medium-sized and acrocentric, whereas the Y chromosome is small and acrocentric, largely heterochromatic as revealed by C-banding. This C-banding also shows that all centromeres have constitutive heterochromatin, with the notable exception of the smallest metacentric pair (9) with only a faint labelling, and the smallest subtelocentric pair (18) with no visible labelling. This pair 18 is also bearing the active NORs. The telomeric FISH labelling is regularly distributed on all telomeres, without an obvious interstitial signal.

The karyotype of a male *Mastomys natalensis* has 2N = 32 chromosomes (Fig. 4). The X chromosome is large and metacentric, whereas the Y chromosome is medium-sized and acrocentric, mainly

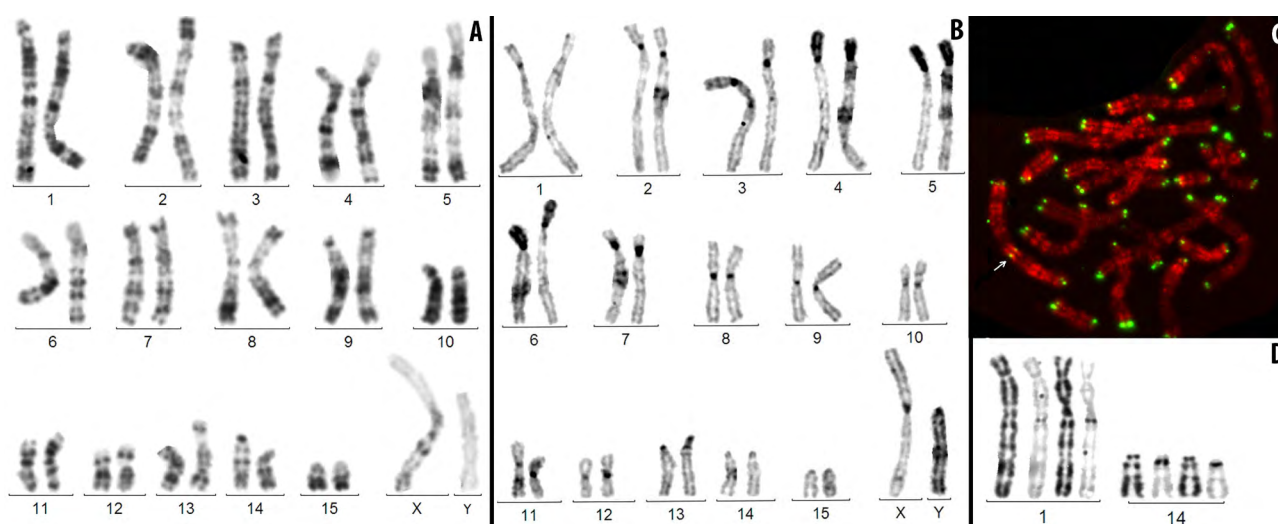


Fig. 4. Karyotype of male *Mastomys natalensis* MNHN-TCCV-841 ($2N = 32, XY$). A) With RBG banding. B) With CBG banding. C) Metaphase plate with telomeric FISH labelling. Arrow indicates the interstitial signal on one chromosome 1. D) Paired NOR-carrier chromosomes 1 and 14 with alternatively RBG banding and Ag-NOR staining.

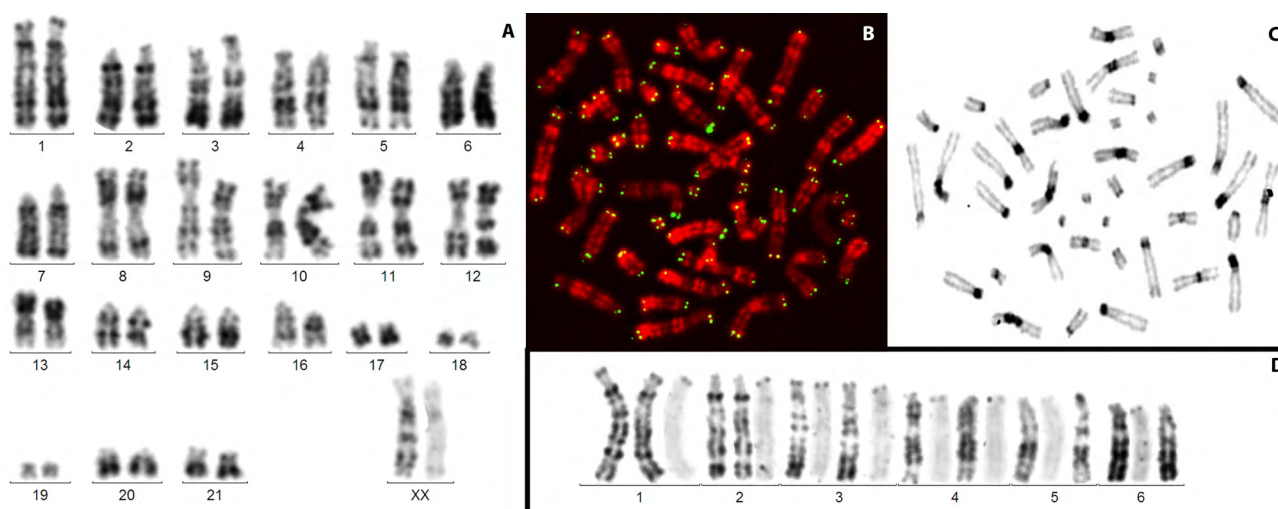


Fig. 5. Karyotype of female *Lemniscomys striatus* MNHN-TCCV-839 ($2N = 44, XX$). A) With RBG banding. B) Metaphase plate with telomeric FISH labelling. C) Metaphase plate with CBG banding. D) Paired NOR-carrier chromosomes 1 to 6 with alternatively RBG banding and Ag-NOR staining (only one NOR-carrier chromosome for pairs 1, 3 and 6).

heterochromatic. The C-banding reveals that constitutive heterochromatin is also distributed as blocks on the centromeric region, on the short arms of three large subtelocentric pairs (4 to 6), and to a smaller extent on the short arm of the X chromosome. The active NORs are located at the centromeric end of acrocentric pair 14, and on a secondary constriction of the long arm of pair 1. A small but clear interstitial telomeric signal (ITS) is also visible near this secondary constriction.

The karyotype of a female *Lemniscomys striatus* has $2N = 44$ chromosomes (Fig. 5). The X chromosomes are large and submetacentric. In order to ensure their correct identification, chromosome painting with a human X chromosome probe was performed: the long arm of each X chromosome

appears homologous to the human X chromosome, whereas the heterochromatic short arm display only a faint signal (Fig. S1A). Heterochromatin is heterogeneously distributed on all centromeres, the larger chromosomes having larger heterochromatic centromeric blocks. The active NORs are distributed at the telomeric end of short arms of one or two chromosomes from pairs 1 to 6. The telomeric labelling is regularly distributed on all telomeres, without an obvious interstitial signal.

The karyotype of a male *Uranomys ruddi* has $2N = 60$ chromosomes (Fig. 6). The X chromosome is metacentric and one of the largest chromosomes in the set, with a large heterochromatic block surrounding the centromere, whereas the Y chromosome is small and acrocentric, mainly

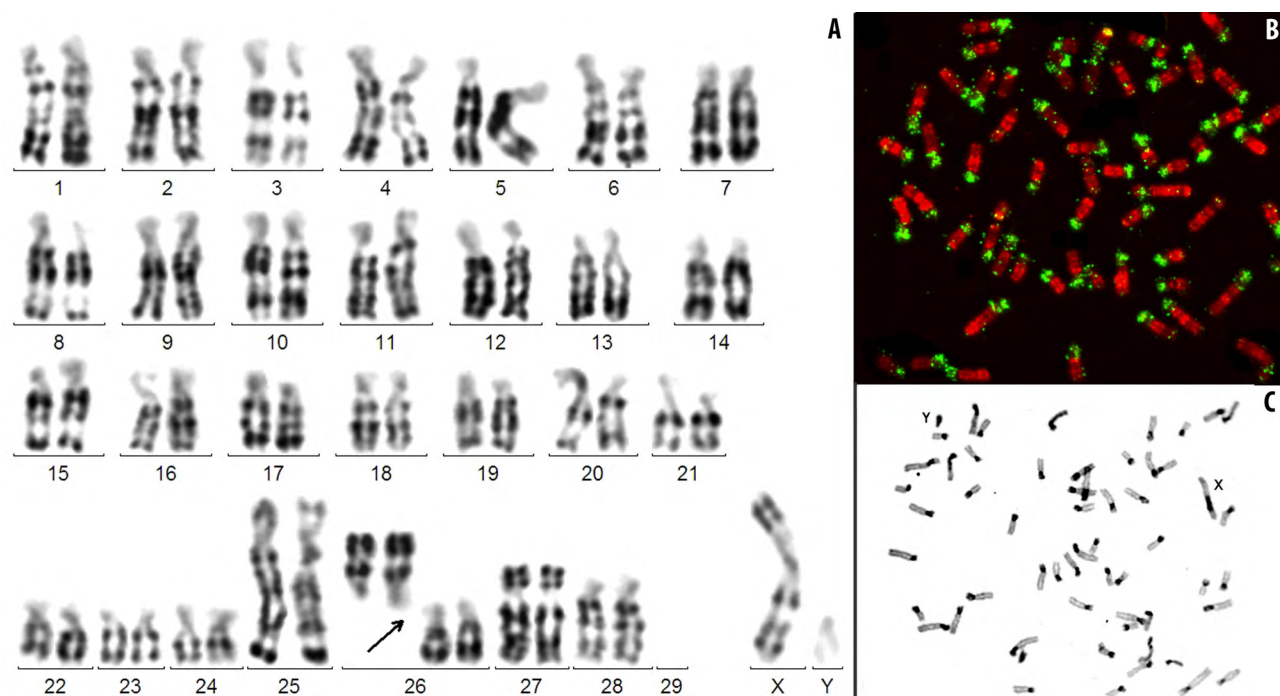


Fig. 6. Karyotype of male *Uranomys ruddi* MNHN-TCCV-833 ($2N = 60, XY$). A) With RBG banding. Arrow indicates two pairs of chromosomes corresponding to the single pair 26 in the karyotype of the same species described by Viegas-Péquignot et al. (1983) for a specimen from Côte d'Ivoire. B) Metaphase plate with telomeric FISH labelling. Most heterochromatic short arms labelled by the signal. C) Metaphase plate with CBG banding. Most of short arms are strongly heterochromatic, X and Y chromosomes are labelled.

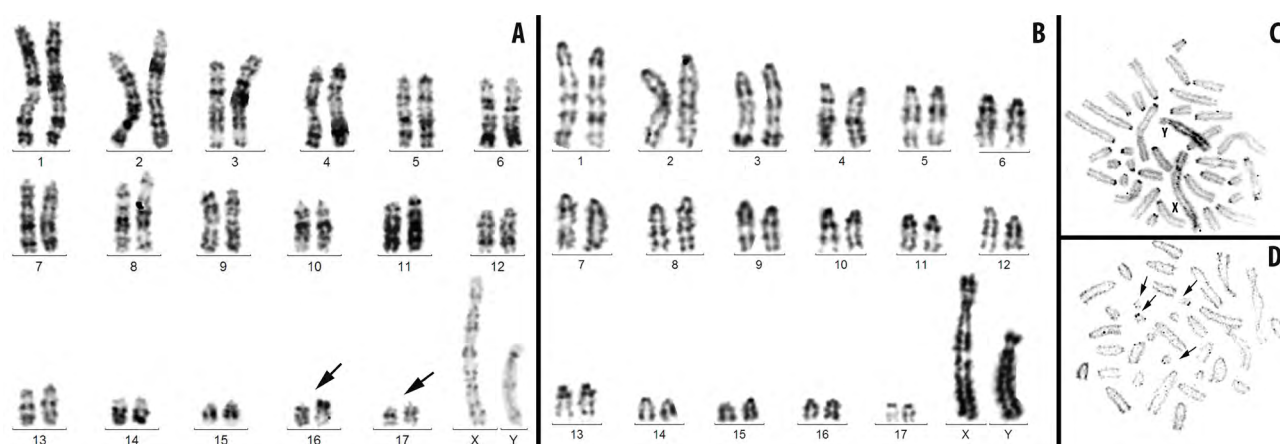


Fig. 7. Karyotype of male *Praomys derooi* MNHN-TCCV-856 ($2N = 36, XY$). A) With RBG banding. Arrows indicate the NOR-carrier chromosome pairs 16 and 17. B) With GBG banding, reciprocal to RBG banding. C) Metaphase plate with CBG banding. X and Y chromosomes are labelled. D) Metaphase plate with Ag-NOR staining. Arrows indicate the NOR-carrier chromosomes 16 and 17.

heterochromatic. The main distinctive feature of this karyotype is that most of the short arms of the autosomes are strongly heterochromatic and almost entirely painted by the telomeric signal.

The karyotype of a male *Praomys derooi* has $2N = 36$ chromosomes (Fig. 7). All chromosomes are acrocentric, except the gonosomes. The X chromosome is large and subtelocentric, whereas the Y chromosome is medium-sized and subtelocentric. The X chromosome is entirely painted by the human X chromosome probe, but a faint signal is also visible on the Y chromosome

(Fig. S1B). The CBG and GBG banding indicate that heterochromatin (late replicating) is distributed as small blocks on all centromeres, on X chromosome and especially on the Y chromosome. The active NORs are located on the two pairs 16 and 17.

The karyotype of a male *Hylomyscus pamfi* has $2N = 46$ chromosomes (Fig. 8). The X chromosome is large and submetacentric, and the Y chromosome is small and acrocentric. These gonosomes present a light heterochromatic marking by CBG banding, with a stronger signal on the long arm of chromosome X. The CBG banding also

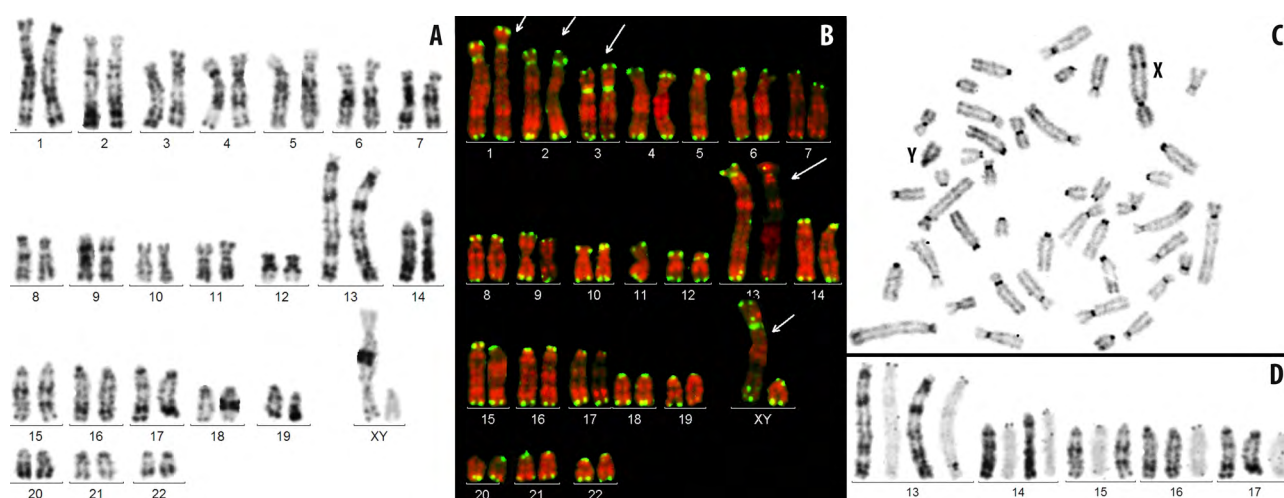


Fig. 8. Karyotype of male *Hylomyscus pamfi* MNHN-TCCV-861 ($2N = 46, XY$). A) With RBG banding. B) With telomeric FISH. Arrows indicate the chromosomes displaying interstitial signals (pairs 1, 2, 3, 13 and chromosome X). C) Metaphase plate with CBG banding. X and Y chromosomes are labelled. D) Paired NOR-carrier chromosomes 13 to 17 with alternatively RBG banding and Ag-NOR staining (only one NOR-carrier chromosome for pair 15, 16 and 17).

indicates that heterochromatin is distributed as small blocks on all centromeres. NORs are located at the telomeric end of short arms of one or two chromosomes from pairs 13 to 17. The telomeric labelling is regularly distributed on all telomeres, but is also present at the centromeres of pairs 1, 2, 3, 13 and chromosome X.

Discussion

Cytogenetic comparisons

Cytogenetic analyses in mammals showed that there is extensive karyotypic diversity among extant species and that many closely related species or even populations possess different karyotypes indicating that chromosomal differentiation often occurs during, or shortly after cladogenesis (Dobigny et al. 2017). Thus, descriptions of mammalian karyotypes serve an important role for characterizing chromosomal rearrangements, which provide information on genetic barriers to gene flow and ultimately on the processes involved in speciation. In this paper we provide cytogenetic data for six of the 12 captured species. For five of them we were able to compare our data with previously published data from the same or closely related species, and the karyotype of the sixth is published here for the first time.

The karyotype of the male *R. rattus* ($2N = 38, XY$) seems identical to the most common form of previously published karyotypes for the black rat from Europe, Africa, America and Western Asia (Yosida 1977, Belcheva & Bisserskov 1984), confirming the globally stable organization of the black rat karyotype in this range.

The other karyotypes already described for *L. striatus* are relatively variable, with a diploid number of 44 or 48 chromosomes, and even more variable autosomal fundamental numbers (Castiglia & Ouge 2008). The female studied here ($2N = 44, XX$) has a karyotype similar to those already published for other specimens from Benin with $2N = 44$, and autosomal fundamental number of 68 or 72-74 (Capanna et al. 1997, Castiglia & Ouge 2008), depending of the number of heterochromatic short arms observed. The short arm of X chromosomes is also heterochromatic, explaining why the human X painting probe mainly covers the long arm of *L. striatus* X chromosomes. The large amount of heterochromatin in *L. striatus* and its dynamics explain the karyotype variability in its distribution range, and may contribute to reproductive isolation between subspecies. Up to 14 subspecies of *L. striatus* were originally described based on the basis of coat colour and cranio-dental morphology, and a deep phylogeographical structure is known in this species based on mitochondrial data (Nicolas et al. 2008). An integrative study combining morphological, molecular and cytogenetic data is now required to review the phylogeography and taxonomy of this species.

The karyotype of the male *U. ruddi* ($2N = 60, XY$) is different from those already published for this species, with $2N = 50$ in Sénégal (Granjon et al. 1992), $2N = 52$ in Central African Republic (Matthey 1970) and $2N = 58$ in Côte d'Ivoire (Viegas-Péquignot et al. 1983). Without banding data, no detailed comparison is possible with the karyotypes from Sénégal and Central African Republic, but we compared our results with the

karyotype from Côte d'Ivoire, and they differ by a fission/fusion event: the metacentric chromosome 26 in the karyotype from Côte d'Ivoire corresponds to two small acrocentric chromosomes in our karyotype from Benin. In Fig. 6A, these two small chromosomes at position 26 represent this. The large amount of heterochromatin on the short arms of autosomes may play a major role in the variation of diploid numbers by fission/fusion events in this species, as visible here for specimens from Benin and Côte d'Ivoire. With this large variability of diploid numbers and the significant variability in morphology already observed across the geographical range of this species (Verheyen 1964), an integrative study combining morphological, molecular and cytogenetic data to review the phylogeography and taxonomy of *Uranomys* specimens from their entire range is warranted.

Mastomys natalensis, *H. pamfi* and *P. derooi* are all belonging to the tribe Praomyini (Aghová et al. 2018). The karyotype of *H. pamfi* ($2N = 46$, XY) has not previously been published. We, therefore, performed a detailed comparison with a specimen of *H. walterverheyeni* from Gabon, present in the TCCV collection (MNHN-TCCV-391), and already described in CBG banding (*H. cf. stella*, specimen number 2001-100 in this article) by Lecompte et al. (2005). The side-by-side comparison of RGB banding and telomeric FISH karyotypes from *H. pamfi* and *H. walterverheyeni* is presented in Fig. S2. Their karyotypes seem nearly identical in RGB banding, but the telomeric FISH reveals that only the X chromosome display ITS in *H. walterverheyeni*, whereas ITS are present on chromosome pairs 1, 2, 3 and 13, corresponding probably to rapid evolution of a class of tandem repeats between the two species.

The karyotype of the male *P. derooi* ($2N = 36$, XY) seems identical to the one previously published for another specimen from Benin (Igbéré locality; Granjon et al. 2005). It is also the same as the karyotypes described for *P. daltoni* from Burkina Faso, Mali and Sénégal (Viegas-Péquignot et al. 1983, Granjon et al. 1992, 2005, Dobigny et al. 2001, Bryja et al. 2010), but differs from the karyotype for *P. daltoni* from Cameroon having $2N = 30$ chromosomes (Bryja et al. 2010). According to Bryja et al. (2010) this diploid number difference ($2N = 36$ for *P. derooi* and *P. daltoni* vs. $2N = 30$ for *P. daltoni* in Cameroon) could be caused by the accumulation of three centric fusions from the

original $2N = 36$ karyotype, but the poor resolution of G-bands prevent its unambiguous confirmation. *Praomys derooi* was described by Van der Straeten & Verheyen (1978) as a small, grey-bellied and commensal species, clearly distinguishable from *P. daltoni*. However, recent genetic data (including our four specimens from Lama forest) showed that *P. derooi* is an ingroup of *P. daltoni* (Bryja et al. 2010, Mikula et al. 2020), and it has been proposed that *P. derooi* should be considered as an ecotype rather than a valid species, and our cytogenetic data are in agreement with this conclusion.

The karyotype of the male *M. natalensis* ($2N = 32$, XY) also seems identical to those previously published from specimens sampled in Sénégal (Duplantier et al. 1990, Britton-Davidian et al. 1995), Chad or Kenya (Volobouev et al. 2002), whereas the inversion and heterochromatin polymorphisms already described are not present in this single animal from Benin. The ITS visible near the secondary constriction bearing NORs on chromosome 1 is an interesting feature, as it could represent a scar from a recent fusion (representing a derived state), while we know that this chromosome has encountered fission or fusion events during the evolution of the tribe Praomyini. By comparison with the *Praomys daltoni* karyotype, Britton-Davidian et al. (1995) and Volobouev et al. (2002) showed that two different fissions of this large chromosome occurred independently in *Mastomys coucha* and *Mastomys erythroleucus*, with a breakpoint in each case near the secondary constriction. The homology between chromosomes 9 and 10 of *M. erythroleucus* and chromosome 1 of *M. natalensis* (named chromosome 3 in Dobigny et al. 2020) has been confirmed by chromosome painting (Dobigny et al. 2010). This *M. natalensis* form of chromosome (not split into two chromosomes) is, therefore, the ancestral state for *Mastomys* and *Praomys* and not a derived state as might be suggested by the ITS visible in *M. natalensis*. By comparison with other species belonging to genera in other rodent tribes (*Mus*, *Rattus* and *Apodemus*), Britton-Davidian et al. (1995) proposed that a previous (older) tandem fusion led to this large chromosome 1 that was ancestral for Praomyini.

Small mammal community composition and conservation

No published records have previously been available for the small mammal fauna of the Lama forest. The present small mammal survey identified



12 species, but our rarefaction analyses show that the true species richness is probably higher. Additional sampling with a higher trapping effort is necessary to get a more complete picture of the small mammal diversity in the Lama forest.

Two species recorded in the Noyau Central (*P. misonnei*, *H. pamfi*) can be considered as closed-forest species, which do not usually colonize Guinea savannahs or other open habitats. *Hylomyscus pamfi* is known only from Benin, Togo and Nigeria (Nicolas et al. 2010b), and was only captured in dense forest. This species is listed as “Data Deficient” by the IUCN Red List because it has only recently been described and, whilst it appears to have a wide distribution, there is still not enough information about its exact distribution, population status, or the threats it faces. The species may be reliant on forest habitat, which is under threat from logging and agricultural expansion (Kennerley 2016). *Praomys misonnei* is widely distributed from eastern Ghana to western Kenya and is only found in forest habitat (Nicolas et al. 2011). It prefers undisturbed rainforest, but may also occur (less frequently) in secondary forest and fallow cultivated habitats within the rainforest (Happold 2013). In the Noyau Central it is the dominant species, where it represents 93% of captures. In African lowland rainforest the small mammal community is usually composed of one or two dominant species and numerous rare species, but the most abundant species generally does not represent more than 60% of captures (Iyawe 1988, Duplantier 1989, Nicolas & Colyn 2003, Barrière et al. 2005, Nicolas et al. 2005, 2009, 2010a, Kennis 2012, Akpatou et al. 2018). Thus, the community structure of the Lama forest is unusual. However during his long-term study in Garmabi forest (Nigeria), Happold (1977) also found that *P. misonnei* can sometimes represents up to 91% of the small mammal species captured each month.

Several species recorded in the Noyau Central are generalist species living in a variety of biotopes (Duplantier et al. 1996, Kan Kouassi et al. 2008, Happold & Happold 2013): *C. fuscomurina*, *M. erythroleucus* and *M. (N.) minutoides*. *Arvicanthis cf. rufinus* is considered as a typical Guinean woodland species (Monadjem et al. 2015). Finally, an open-habitat and/or commensal species, *M. natalensis*, was also captured in the Noyau Central in low abundance (2 specimens from 252 captures).

In Koto village and the crops around this village, several open-habitat and/or commensal species were captured (*C. cf. olivieri*, *L. striatus*, *M. natalensis*, *P. derooi*, *R. rattus*, *U. ruddi*). Despite our low trapping effort in crops and houses, the specific richness appears relatively high in this habitat, and additional sampling is clearly needed to obtain reliable estimates of species richness.

In conclusion, despite the Noyau Central being composed of a mosaic of habitats our results show that it still supports a small mammal fauna typical of native forest. Additional sampling methods (e.g. canopy trapping, other types of traps) and long-term surveys will be necessary to complete our species list, but our data show that the overall ecological conditions of the Noyau Central appear to meet the habitat requirements of at least several forest rodent species. These results are congruent with those obtained on other animal groups, and highlight the importance of the Lama forest, one of the largest remnants of forest in Benin, for the conservation of the country's forest biodiversity (Waltert & Mühlenberg 1999, Kassa & Sinsin 2003). It could be argued that given the rapid loss of forest in West Africa the small mammal fauna sampled in the Noyau Central in 2007 is not representative of the actual fauna. Time-series analysis of Landsat images characterizing forest extent and change (Hansen et al. 2013) show important forest loss from 2000 to 2018 in the teak plantations surrounding the Noyau Central, particularly at the southern region of the Lama forest reserve, but with almost no forest loss in the Noyau Central (Fig. 1). It is highly probable that the conditions in the Noyau Central still allow the persistence of forest and generalist species within it. However, the loss of forest around the Noyau Central may have facilitated the penetration of open-habitat and/or commensal species within it. Small mammal sampling is required to examine this possibility. Finally, it is important to underline that the Dahomey Gap region is not only an area of contact between the Upper Guinean and Lower Guinean fauna, but that it also harbours its own endemic fauna. In the Lama forest we captured one rodent species endemic to this region (*H. pamfi*), and the same result was obtained for several other animal species or subspecies (Sinsin et al. 2002, Goergen 2003, Ullenbruch 2003, Nagel et al. 2004) reinforcing the importance of the Lama forest for biodiversity conservation in the region.



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Supplementary online material

Fig. S1. Metaphase plate with human X chromosome painting. A) *Lemniscomys striatus* female MNHN-TCCV-839. Arrows indicate the two X chromosomes, with strong painting on the long arms and faint painting on the heterochromatic short arms. B) *Pramomys derooi* male MNHN-TCCV-856. Arrows indicate respectively the X chromosome with strong painting and the Y chromosome with faint painting. C) *Hylomyscus pamfi* male MNHN-TCCV-861. Arrow indicates the X chromosome (<https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-2-2020-Nicolas-et-al.-Fig.-S1.jpg>).

Fig. S2. Comparison of karyotypes between a female *Hylomyscus walterverheyeni* (MNHN-TCCV-391) and a male *Hylomyscus pamfi* (MNHN-TCCV-861). Each composite karyotype represents one chromosome for *H. walterverheyeni* (left) and one chromosome for *H. pamfi* (right) per pair. Both XX chromosomes from *H. walterverheyeni* (left) and XY chromosomes from *H. pamfi* (right) are represented A) with RBG banding. B) with telomeric FISH. Arrows indicate the chromosome of *H. pamfi* displaying interstitial signals (pairs 1, 2, 3, 13 and chromosome X) (<https://www.ivb.cz/wp-content/uploads/JVB-vol.-69-2-2020-Nicolas-et-al.-Fig.-S2.jpg>).